45. The method of claim 16, wherein the physiologically acceptable gas is C_2F_6.

46. The method of claim 16, wherein the physiologically acceptable gas is C_4F_8.

47. The method of claim 16, wherein the physiologically acceptable gas is C_4F_{10}.

48. The method of claim 16, wherein the physiologically acceptable gas is SF_6.

49. (New) The method of claim 1, in which at least part of the phospholipids are in the form of liposomes.

50. (New) The method of claim 15, in which at least part of the phospholipids are in the form of liposomes.

REMARKS

Claims 1-3, 7, 13-22, 26, and 30-48, and new claims 49 and 50 are pending in this application.

Applicants note that new claims 49 and 50, which have been added with this Amendment and Response, were previous claims 6 and 25 which were inadvertently and incorrectly included in the cancellation instruction in the Amendment filed December 7, 2000, but were included as part of the pending claims in Applicants' Amendment filed October 25, 2001. Since the Examiner indicated in the Office Action that claims 6 and 25 were cancelled and cannot simply be reinstated, Applicants have thus added the inadvertently cancelled claims 6 and 25 back into the application as new claims 49 and 50.

In the Office Action, Applicants' pending claims have been rejected under 35 U.S.C. § 103 over Glajch, and over Cerny and Ryan, in view of Glajch, Quay, Tickner and the DuPont bulletin. Applicants respectfully traverse.

Applicants believe that these claims are patentable for the same reasons that the original claims were patentable as explained in Applicants' March 29, 2000 Amendment And Response

To Office Action, December 7, 2000 Preliminary Amendment and October 25, 2001 Amendment. They are supported in the specification as shown in Applicants' Preliminary Amendment filed July 15, 1998, and Applicants are not aware of any prior art that has all of the elements of the claims or which in proper combination with other prior art would provide all of the elements of the claims.

Applicants understand that the Examiner is aware that U.S. Patent No. 5,413,774, the parent patent from which this '963 reissue application was filed, is involved in Interference No. 103,880. Applicants further understand that the Examiner is also aware of other pending, resolved or requested interferences relating to the general subject matter of this application and, thus, Applicants encourage the Examiner to consider them as well.

I. Applicants' Claimed Stabilized Microbubbles And The Proven Unexpected Results

Applicants' stabilized microbubbles were first disclosed in EP 90810262.7, filed April 2, 1990. However, since Applicants are not required to rely on that date to overcome the cited references, the issue of the effective filing date (determined by Examiner to be January 24, 1992) is moot.

Applicants were the first inventors to discover that the combination of Applicants' claimed fluorinated gas stabilized by phospholipids in lamellar or laminar form at the gas/liquid interface gave greater than expected stability and contrast duration.

Specifically, Applicants discovered that contrast agents containing Applicants' claimed fluorinated gas gave superior imaging results compared to other known gases such as air or nitrogen because the contrast agents remained longer in the bloodstream than known contrast agents made with other gases. Proof of the superiority and nonobviousness of the Applicants' stabilized microbubble invention is explicitly disclosed in the examples of the specification. *See*

Examples 4-6. Applicants have even proven with experimental evidence that their claimed stabilized microbubbles have critical pressures nearly twice as great and have contrast durations nearly four times longer than the closest prior art gas of air (*i.e.*, stabilized microbubbles of air). *Id.*

In further support of unexpected results of Applicants' claimed invention, Applicants also concurrently submit as Exhibit 1 the Declaration Of Michel Schneider, Ph.D which contains additional experimental evidence establishing, as per the Examiner's request, an unexpectedly superior trend commensurate with the scope of Applicants' claimed stabilized microbubbles containing Applicants' claimed fluorinated gases and the closest prior art gas (*i.e.*, stabilized microbubbles with air). Specifically, Dr. Schneider performed laboratory experiments which showed that phospholipid stabilized microbubbles containing well known freon gases CF_4, C_2F_6, C_2ClF_5, C_3F_8, and C_4F_8 (cyclic) have far superior half-life, area under the curve, and persistence measurements in the left ventricle of the heart compared to phospholipid stabilized microbubbles containing air. Exhibit 1, Declaration of Michel Schneider, Ph.D., ¶¶ 4-10.

With such proof of unexpected and superior results, Applicants' claimed invention is clearly nonobvious with respect to the cited references and the Examiner is respectfully requested to withdraw this rejection under 35 U.S.C. § 103. MPEP § 716.02(a). Applicants note that these results were submitted in related U.S. application Serial No. 09/401,838 and accepted by the Examiner as evidence supporting the nonobviousness of the claimed invention, and thus Applicants respectfully submit that the same patentable result should be found in this case.

Furthermore, as discussed below, none of the cited references, viz, Glajch, or Cerny and Ryan, in view of Glajch, Quay, Tickner and the DuPont bulletin, may be combined to disclose the Applicants' claimed stabilized microbubble invention. Specifically, there is no proper

combination of these references which will disclose or suggest anywhere the limitation wherein Applicants' claimed fluorinated gas is "bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface."

II. Applicants' Claimed Microballoons And Surprising Results

Applicants' microballoons were first disclosed in EP 90810367.4, filed May 18, 1990. However, since Applicants are not required to rely on that date to overcome the cited references, the issue of the effective filing date (determined by Examiner to be January 24, 1992) is moot.

Applicants were the first inventors to discover that the combination of Applicants' fluorinated gas with Applicants' claimed organic polymer envelope gave greater than expected stability and contrast duration.

Prima facie proof of the superiority and nonobviousness of the Applicants' microballoon invention are explicitly disclosed in the examples of the specification. *See* Examples 1, 2. Applicants have even proved with experimental evidence that their claimed microballoons have critical pressures more than twice as great and have contrast durations more than five times longer than the closest cited prior art filled albumin microballoons. *Id.*

Further proof is shown by the commercial products Albunex®, which is air filled albumin microspheres, and Optison®, which has the same albumin microsphere as Albunex® but its microspheres are filled with the fluorinated gas C_3F_8. Applicants understand that Albunex® failed as a commercial product because it was not efficacious enough while Optison® has succeeded because it has been shown to be efficacious. Numerous articles confirm the superiority of Optison® over Albunex®. *E.g.*, Podell et al, "Physical and biochemical stability of Optison®, an injectable ultrasound contrast agent", 30 Biotechnol. Appl. Biochem., pp. 213-223 (1999)(see especially pp. 220-222); Clark et al, "Cardiac Imaging Using Optison," A Symposium: Advances In Echocardiography, p. 14G (2000); Hancock, et al., "Evaluation of

myocardial, hepatic, and renal perfusion in a variety of clinical conditions using an intravenous ultrasound contrast agent (Optison®) and second harmonic imaging," 81 Heart, pp. 636-641 (see especially p. 640) (1999) (all included as Exhibit 2). This is further proof of the unexpected surprise of Applicants' invention.

With such clear proof of unexpected and superior results, Applicants' claimed invention is clearly nonobvious with respect to the cited references and the Examiner is respectfully requested to withdraw this rejection under 35 U.S.C. § 103. MPEP § 716.02(a). Applicants note that these evidence were submitted in related U.S. application Serial No. 09/740,653 and accepted by the Examiner as evidence supporting the nonobviousness of the claimed invention, and thus Applicants respectfully submit that the same patentable result should be found in this case.

Furthermore, none of the cited references, viz, Glajch, or Cerny and Ryan, in view of Glajch, Quay, Tickner and the DuPont bulletin, may be combined to disclose the Applicants' claimed microballoon invention. Specifically, there is no proper combination of these references which will disclose or suggest anywhere the limitation wherein Applicants' claimed fluorinated gas is "bounded by an organic polymer envelope at the gas/liquid interface, said polymer envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids."

III. Applicants' Claims Are Patentable Over The Cited References

Applicants' pending claims have been rejected under 35 U.S.C. § 103 as allegedly being obvious over Glajch, or over Cerny or Ryan in view of Glajch, Quay, Tickner and the DuPont bulletin. Applicants respectfully traverse.

Applicants respectfully submit that their claims are patentable and nonobvious over the cited references because there is no motivation to combine them, and even if there was such motivation (Applicants submit there is not), the combination of these references fail to teach or suggest each of the limitations in the Applicants' claims. A brief review of the cited references is provided below:

A. The Cited References

1. Cerny (U.S. Patent No. 4,957,656)

Cerny teaches a continuous sonication method for preparing albumin "microspheres" filled with air. Nitrogen, oxygen, and carbon dioxide may also be used. Cerny states that it solved the prior art manufacturing problem with respect to albumin "microspheres" by disclosing a method which produces "microspheres" on a continuous high production basis. Cerny further states that its "microspheres" are small, stable, and adequate for its uses. Col. 3, lines 36-40. Cerny does not suggest that its method or "microspheres" have any problems that need to be solved or improved. Furthermore, none of the other contrast agents cited by the Examiner may be produced using Cerny's methods. Thus, Cerny teaches away from combining its teachings with any of the other references cited by the Examiner.

2. Ryan (U.S. Patent No. 4,900,540)

Ryan discloses a liposome with an aqueous interior which contains gas or gas precursor. Col. 2, lines 15-17, 51-52. Thus, the interior of the Ryan liposome is a mixture of water and gas. Hence, Ryan does not disclose gas filled microbubbles or microballoons. Contrary to the Examiner's arguments, the term "gas filled" as used in Applicants' claims excludes microvesicles which contain liquid, such as the Ryan liposomes.

Furthermore, Ryan teaches that its liposomes are adequate for its uses and does not suggest any problems that need to be solved or any improvements that need to be made. Col. 2,

lines 28-30. In fact, Ryan suggests that liposomes provide the most practical and effective encapsulation for aqueous materials (col. 1, lines 27-29), thereby distinguishing and teaching away from combining with other types of contrast agents such as those disclosed in the art cited by the Examiner.

3. Glajch (U.S. Patent No. 5,147,631)

Glajch discloses microparticles made of inorganic material. These microparticles are porous and may be crystalline. These microparticles are not a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface, nor are they an organic polymer envelope at the gas/liquid interface, said envelope formed from one or more polymers selected from the group consisting of polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids. Thus, Glajch cannot alone or in combination with any other reference, render Applicants' claimed invention obvious.

Furthermore, despite the Examiner's reliance on Glajch for disclosing the use of CF_4 and C_2F_6 gases, Glajch does not suggest anywhere that its gases can be used, or would even be useful with other contrast agents. Glajch does not even indicate anywhere that the type of gas used affects the imaging performance of the contrast agent. Rather, Glajch states that the key to a viable contrast agent is good mechanical stability and rigidity (col. 4, lines 50-54), and that the inorganic porous particles provide the contrast for ultrasound imaging. Col. 3, lines 3-4. Glajch further teaches its belief that the most desirable contrast effects are obtained by changing the shape, size or porosity of the particle. Col. 4, lines 56-59.

In fact, Glajch specifically distinguishes its invention from the albumin microspheres of Feinstein (similar to Cerny), from the saccharide particles of Tickner Col. 1, lines 39-54. Thus, by teaching that its inorganic particles are superior to other types of contrast agents, and by

suggesting that it has solved all the problems with the mechanical stability and rigidity of the prior art, Glajch thus teaches away from the microballoons of Applicants' claims as well as from any combination with the prior art cited by the Examiner.

4. Quay (U.S. Patent No. 5,393,524)

Quay discloses free gas microbubbles which do not have any type of envelope surrounding the gas. The Examiner relies on Quay for disclosing SF_6 and C_4F_8 gases.

However, contrary to the Examiner's statement, Quay at col. 7-8 specifically distinguishes his invention from and criticizes the prior art inventions which may contain shell type materials such as liposomes and albumin "microbubbles". Thus, the Examiner improperly relies on combinations that Quay explicitly rejects as inferior. Furthermore, Quay also states that its free gas microbubbles have "novel and superior" properties compared to other types of contrast agents. *E.g.*, col. 1, line 18; col. 2, lines 17-21. By clearly distinguishing its invention from other contrast agents such as liposomes or albumin "microbubbles", teaching that its free gas microbubbles are superior, and claiming that it has solved all of the problems of prior free gas microbubbles, Quay thus teaches away from combining with any of the references cited by the Examiner.

5. Tickner (U.S. Patent No. 4,265,251)

Tickner discloses the use of saccharide microparticle precursors which dissolve in the bloodstream to release free gas microbubbles. These saccharide microparticles are porous, crystalline, rigid, and preferably ground. The Examiner appear to rely on Tickner for teaching use of fluorinated gas.

However, there is nothing in Tickner which suggests the desirability of using its gas with any other contrast agents. Rather, Tickner states that its method is advantageous and solves the problems of the prior art by providing a solid precursor which dissolves to release free gas

microbubbles. Col. 2, lines 23-36. Tickner does not suggest any problems with its invention that need to be solved or any improvements that need to be made. Thus, Tickner teaches away from any combination with the prior art cited by the Examiner.

6. DuPont Technical Bulletin

The DuPont Bulletin discloses a list of gases which are freons. To the extent the Examiner appear to rely on DuPont to verify anything other than that the term "freon" includes the gases in the Applicants' claims, such reliance would be improper because there is no indication in DuPont whatsoever that these gases may be used as contrast agents.

B. There Is No Motivation To Combine

The references relied upon by the Examiner fail to provide the necessary incentive or motivation to combine them in an attempt to create the Applicants' claimed invention. There is nothing in any of the references to suggest the desirability of the combination or modification in the manner indicated by the Examiner. Thus, the combination of references proposed by the Examiner is improper and Applicants respectfully request that this rejection be withdrawn.

1. There Is No Suggestion In The Cited References To Combine

It is well-established that before a conclusion of obviousness may be made based on a combination of references, there must have been a reason, suggestion, or motivation to lead one of ordinary skill in the art to combine those references. *In re Dembiczak*, 50 U.S.P.Q.2d 1614, 1617-18 (Fed.Cir. 1999)("Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references.")

Merely asserting that it would have been within the skill of the art to substitute one type of gas for another in the contrast agent of the primary reference is not enough. *In re Fine*, 5

U.S.P.Q.2d 1596 (Fed.Cir. 1988)(Holding that there was no support for the Examiner's mere assertion that it would have been obvious to substitute one type of detector for another in the system of the primary reference); *In re Jones*, 21 U.S.P.Q.2d 1941 (Fed.Cir. 1992)(Holding that there was no suggestion to combine a primary herbicide reference with secondary references directed to shampoo additives or byproducts of mopholines to arrive at the claimed invention.); MPEP § 2143.01.

There is nothing in any of the cited references to suggest the desirability of the combination or modification in the manner indicated by the Examiner. In fact, each of the references Cerny, Ryan, Glajch, Quay and Tickner teach against the modification of their invention or the combining of parts of their invention with other references because they each teach their belief that their agents are superior to all others, with no problems or improvements needed or even suggested. It is especially significant that despite the presence of the DuPont reference, none of these contrast agent references chose to incorporate or adopt the DuPont teaching regarding gases. The Examiner's proposed combination thus would not have been made by one of ordinary skill in the art. Moreover, there are no road signs or blaze marks in the references that would lead one to ignore the bulk of their teachings and recommendations and be led to anything like Applicants' specific gases, specific stabilized microbubbles and specific polymer microballoons.

2. The Mere Fact That References Can Be Modified Or Combined Is Not Enough

Further, as stated by the Court in *In re Fritch*, 23 U.S.P.Q.2d 1780, 1783-1784 (Fed. Cir. 1992)(emphasis added):

> The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification.

Thus, the mere fact that references can be combined or modified (Applicants believe they cannot be) does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 16 U.S.P.Q.2d 1430 (Fed.Cir. 1990); MPEP § 2143.01. Hence, the Examiner's attempt to combine the cited references alone without any suggestion in the references of the desirability of the modification is improper and should be withdrawn.

3. The Modification Cannot Change The Principle Of Operation Of A Reference

The proposed modification cannot change the principle of operation of a reference. *In re Ratti*, 123 U.S.P.Q. 349 (C.C.P.A. 1959); MPEP § 2143.01. However, the Examiner's proposed modification would effectively change the principle of operation of each of the references. For example, Quay ("free gas microbubbles") cannot be combined with Cerny (albumin "microspheres") or Ryan (liposomes) because Quay explicitly teaches the benefits of having no surrounding materials while Cerny and Ryan both rely on the type of surrounding material for stability. Thus, the Examiner's proposed modification is improper and this rejection should be withdrawn.

4. There Is No Reasonable Expectation Of Success

There also must be a reasonable expectation of success from the prior art in combining the references. *In re Vaeck,* 947 F.2d 488, 20 U.S.P.Q.2d 1438, 1442 (Fed.Cir. 1991). This motivation to combine and the reasonable expectation of success both must be found in the prior art and not the Applicants' disclosure. *In re Vaeck,* 20 U.S.P.Q.2d at 1442. Using the Applicant's own disclosure in an obviousness analysis is considered improper and prohibited by case law. *Grain Processing Corp. v. American Maize-Products Co.*, 840 F.2d 902, 907, 5 USPQ2d 1788, 1792 (Fed. Cir. 1988)("Care must be taken to avoid hindsight reconstruction by

using 'the patent in suit as a guide through the maze of prior art references, combining the right references in the right way so as to achieve the result of the claims in suit.'"); *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988)("One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention.")

Since each of the cited references are directed to different types of ultrasound contrast agents, there is thus no reasonable expectation of success that the agents taught by Cerny (albumin "microspheres"), or Ryan (liposomes with an aqueous interior which contains gas) will work with the gases disclosed in Glajch (inorganic microparticles), Quay (free gas microbubbles), Tickner (free gas microbubbles in saccharide microparticles) and DuPont (not for ultrasound contrast agents) as suggested by the Examiner. This conclusion is supported by the assertion by each reference of its respective superiority over the other types of contrast agents and distinction from them.

Without any reasonable expectation of success, it is improper to combine the references cited by the Examiner and withdrawal of this rejection is respectfully requested.

C. The Cited References Do Not Render Applicants' Claims Obvious

Even if there exist some motivation to combine, which Applicants assert there is not, all of the claim limitations must still be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 U.S.P.Q. 580 (CCPA 1974); In re Wilson, 424 F.2d 1382, 1385, 165 U.S.P.Q. 474, 496 (CCPA 1970). MPEP § 2143.03.

Contrary to the Examiner's statement, attempting to properly modify the invention of Cerny or Ryan to substitute the gases therein with the gases of Glajch, Quay, Tickner or the

DuPont bulletin would still fail to teach or suggest each of the limitations in the Applicants' claims.

1. Applicants' Claimed Stabilized Microbubbles

Cerny, Ryan, Glajch, Quay, Tickner and DuPont do not in any proper combination, teach, suggest, or disclose the method of making the Applicants' stabilized microbubbles wherein Applicants' claimed fluorinated gas or gas mixture is bounded by a stabilizing layer of one or more film forming phospholipids in lamellar or laminar form at the gas/liquid interface. The method of making Applicants' stabilized microbubbles are claimed in claims 1-3, 6, 7, 13-15, 18, 21, 25, 26, 32, 35, 37, 38-42, 49 and 50.

Additionally, Cerny, Ryan, Glajch, Quay, Tickner and DuPont do not in combination, teach, suggest, or disclose forming the Applicants' stabilized microbubbles by the gas substitution method of claims 2, 3, 18, and 21.

2. Applicants' Claimed Microballoons

Cerny, Ryan, Glajch, Quay, Tickner and DuPont also do not in any proper combination, teach, suggest, or disclose the method of making Applicants' microballoons wherein Applicants' claimed fluorinated gas or gas mixture is bounded by an organic polymer envelope at the gas/liquid interface, the envelope formed from one or more polymers selected from polylactic or polyglycolic acid and their copolymers, denatured albumin, reticulated hemoglobin, and esters of polyglutamic and polyaspartic acids. The method of making Applicants' microballoons are claimed in claims 16, 17, 19, 20, 22, 30, 31, 33, 34, 36, 43-48.

Additionally, Cerny, Ryan, Glajch, Quay, Tickner and DuPont do not in combination, teach, suggest, or disclose forming the method of forming Applicants' microballoons by the gas substitution method of claims 19, 20, and 22.

IV. Conclusion

For the reasons stated above, because pending claims 1-3, 6, 7, 13-22, 25, 26, 30-50 are fully supported in the specification and are fully patentable over any references cited, favorable action on these claims is requested.

If there are any questions, the Examiner is respectfully asked to contact the Applicants' attorney.

Respectfully submitted,

NIXON & VANDERHYE, P.C.

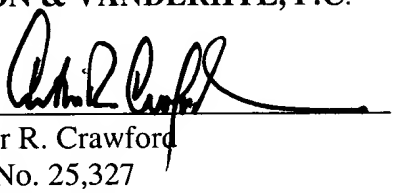
By: 

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Table 1: *In vivo* characteristics and persistence of phospholipid-stabilized gas microbubbles

	Left Ventricle (n=4)			
	1 max Pixels	Area under the curve	Half-life sec	Persistence sec
Air	37 ± 8	451 ± 165	9 ± 2	12 ± 5
CF_4	42 ± 6	1052 ± 261	29 ± 8	22 ± 2
C_2F_6	50 ± 7	4516 ± 1186	82 ± 18	91 ± 9
C_2ClF_5	57 ± 6	3431 ± 497	65 ± 5	53 ± 9
C_3F_8	47 ± 5	3798 ± 410	86 ± 5	85 ± 6
C_4F_8cyc	41 ± 4	2717 ± 1021	59 ± 21	64 ± 16